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50 YEARS AGO

Bacterial transformation

In the 1920s pneumonia was a serious illness. With no antibiotics to turn to, mortality was high and medical officers of health responsible for the public health of local communities frequently encountered the disease. One such was Dr J. Bell Ferguson, Medical Officer of Health for Smethwick in the English 'Black Country'. Inevitably Bell Ferguson was in contact with the British Ministry of Health's central Pathological Laboratory, on the staff of which was Dr Fred Griffith, who, between April 1920 and January 1922, had made a survey of the different serological types of pneumococci in samples from 150 cases of lobular pneumonia and had published the results, a Ministry of Health report, in 1922.

Between February 1922 and March 1927, Bell Ferguson supplied Griffith with many specimens, chiefly sputum but occasionally lung tissue, from Smethwick people who had contracted lobular pneumonia. During the course of other inquiries and routine examination of sputum, which diverted him from a full-time investigation of pneumococci, Griffith slowly accumulated further data about the changing incidences and comparative pathogenicity of the different serological types of pneumococci in the Smethwick samples. By 1927 he felt that at last he had sufficient new information to make a worthwhile contribution to the literature and towards the end of August 1927 he submitted his findings of the past 5 years to the *Journal of Hygiene* [1]. The paper, all 47 pages of it, was published in January 1928; the gestation period had been long but the results were worth waiting for because Griffith had discovered bacterial transformation.

Clearly no ordinary medical microbiologist, Griffith was concerned to understand the origin of epidemic types of pneumococci. In his survey of the incidence of different serotypes in the samples from Smethwick he had noticed that between 1922 and 1927 the incidence of Type II pneumococci had fallen from 32.6 to 7.4% while the incidence of Group IV cocci had risen from 30.0 to 53.7%. He also noticed repeatedly that the serotypes present in sputum samples from individual patients taken over a period of days or, if they survived, weeks, varied and that attenuated strains appeared.

Observations of this sort led Griffith to study *in vitro* attenuation which he achieved by growing virulent bacteria in homologous immune serum. The attenuated colonies that appeared had a distinct morphology; the attenuated R cells had a rough surface because they lacked the smooth carbohydrate capsule of the virulent S strains and, therefore, also lacked detectable amounts of type-specific antigen. He reversed attenuation by passaging R cells in the peritoneal cavity of mice where reversion to the parental S strain sometimes occurred, but the event was 'infrequent and apparently haphazard'. Different R strains reverted to S strains at different frequencies and with some it was possible to obtain reversion by injecting large doses of the R form under the skin of a mouse.

Griffith's epoch-making experiment

These findings led Griffith to his epoch-making experiment, for he reasoned that the more readily reverting R strains had retained 'in their structure a remnant of the original S antigen insufficient in ordinary circumstances to enable them to exert a pathogenic effect in the animal body. When a strain of this character is inoculated in a considerable mass under the skin, the majority of cocci break up and the liberated S antigen may furnish a pabulum which the viable R pneumococci can utilize to build up their rudimentary S structure'. In Griffith's mind, of course, the pabulum was the carbohydrate antigen itself and to prove it he decided to inoculate subcutaneously in four mice a large concentration of heat-killed virulent Type II pneumococci together with the living attenuated pneumococci derived from Type II. The former would, he supposed, provide a large amount of carbohydrate pabulum for the latter to incorporate into a capsule. All four died of a resulting bacteremia in 3–4 days and virulent S type II cocci could be easily isolated from their blood. Controls injected with the living R type or the dead S type cocci remained healthy.

Griffith then repeated the experiment using combinations of dead S type and living R type bacteria of the various serotypes and found, for example, that indeed dead S type III bacteria transformed living R type I or type II bacteria into virulent S type III bacteria. He speculated that the differences in the efficiency of transformation with different serotypes that he observed reflected differences in the amounts and stabilities of their capsular antigens.

Griffith did not pursue the subject further. As his introduction to his 1928 paper makes clear, since 1922 for him it had been only a sideline. But the importance of his results was certainly not lost on his contemporaries; whereas Griffith had spent 5 years making and checking his seminal discovery, within the year of its publication, F. Neufeld and W. Levinthal [2] in Germany had not only confirmed the central observation of the transformation of living pneumococci by dead ones, but also had published the fact. Soon afterwards, in 1930 M. H. Dawson [3] at the Rockefeller Institute published further confirmation, and in 1932 with R. H. P. Sia [4], he reported for the first time transformation *in vitro*. There followed further periodic reports of the transformation of pneumococci, culminating in 1944 in the publication by O. T. Avery, C. M. MacLeod and M. McCarty [5] of irrefutable evidence that Griffith's pabulum was nothing more nor less than DNA, the genetic material.

Ever since bacterial transformation was discovered there has been much speculation and some experimentation about the generality or otherwise of the phenomenon, and in particular whether or not eukaryotic cells can be transformed other than by the DNA of tumour viruses. It is, therefore, a very happy coincidence that in this year, the 50th anniversary of Griffith's publication, G. R. Fink and his colleagues [6] at Cornell University have for the first time shown beyond dispute that transformation is not restricted to bacteria but can also be achieved with yeast, and that the transforming DNA once integrated into the yeast genome behaves as a simple Mendelian element. Furthermore, Fink *et al.* [6] have proved that by transformation, bacterial genes as well as homologous yeast genes can become a stably inherited component of the yeast genome.

Now that recombinant DNA technology makes it feasible to isolate large amounts of virtually any gene from any source we shall, no doubt, be hearing a great deal more about the generality and exploitation of the phenomenon Griffith discovered through astute and painstaking work in the 1920's. JOHN TOOZE

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